Hb-A_2

Chromatographic – spectrophotometric determination
of Hemoglobin A_2
in blood

20 tests **REF KR06-20**

INTENDED USE

Kit for quantitative *in vitro* determination of Hemoglobin A_2 in blood.

PRINCIPLE

Total hemoglobin is adsorbed on an ionic exchange cellulose, balanced with a proper buffer. Interfering substances are separated by washing, hemoglobin A_2 is selectively eluted and assayed spectrophotometrically.

REAGENTS AND MATERIALS

Kit components:	REF KR06-20
REAGENT 1 Hemolyzer	**1 x 21 ml**
REAGENT 2 Tris buffer	**2 x 60 ml**
ATTENTION: contains sodium azide, handle with care.	
COLUMNS Chromatographic columns	**20**

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 20-25°C, sealed reagents and columns are stable up to the expiration date on the label.

SAMPLE

Venous blood collected with heparin or EDTA.
Collect few blood drops from a capillary tube or use the contents of a hematocrit tube.
STABILITY: at least one week at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength:	414 nm
Optical path:	1 cm
Reading:	against distilled water
Temperature:	20-25°C
Method:	spectrophotometric
Recovery:	100 %
C.V.:	2.5 %

PREPARATION OF THE COLUMN

Remove the upper cap and break the bottom tip. With a Pasteur pipette round tip, push the upper filter on the resin. Let the liquid flow completely into the column.

PREPARATION OF THE SAMPLE

Pipette into a tube:

Blood	100 µl
Reagent 1	1.0 ml

Mix well and let it stand for 10 minutes.

CHROMATOGRAPHIC SEPARATION

Pipette into a column:

Hemolyzed solution	50 µµl	discard the eluate
Reagent 2	0.5 ml	discard the eluate
Reagent 2	5.0 ml	collect the eluate

Mix well and read the absorbance of S1 (AS1) solution at 414 nm against distilled water. Pipette into a tube:

Distilled water	10.0 ml
Hemolyzed solution	0.02 ml

Mix well and read the absorbance of S2 (AS2) solution at 414 nm against distilled water.

CALCULATION

% Hb-A_2 = (AS1 / AS2) x 20

REFERENCE VALUES

normal values	1.7 - 3.5	% total Hb
ß-thalassemia	4 - 10	% total Hb

NOTES

1. Hemoglobin S can interfere and give false high values.
2. The upper porous sheet is placed horizontally, in contact with the resin. If not, replace in the original position using a glass rod or the flat tip of a pipette.
3. AS2 absorbance does not usually cause any problem in the determination. When AS2 values are lower than 0.150, false low values of AS1 may result. Calculated percentages could be easily influenced by small variations into the reading. In this case, it is preferable to repeat the determination by preparing a more concentrated hemolysate or by checking the absorbance value before starting chromatography.

REFERENCE

1. L.G. Morin, “Clin. Chem.” 22-2036 (1976)

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	read instructions for use

IVD CE

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